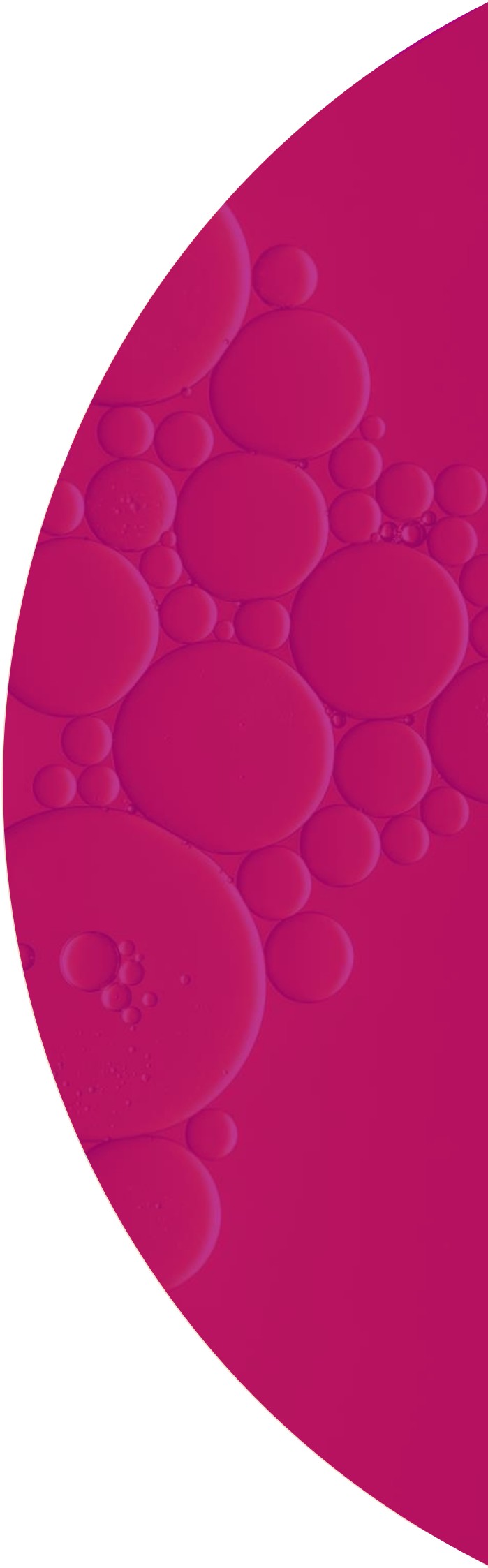
PacBio

Short read eliminator (SRE) kit family

Guide & overview



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Prior to starting

Create 70% EtOH wash buffer by diluting ethanol (96–100%) with DI water.

Storage

All buffers should be stored at room temperature (15–30°C).

Product use

SRE family of kits (SRE XS, SRE, and SRE XL) are intended for research use only.

User supplied equipment and reagent list

Equipment	Model
SRE XS kit	PacBio® (102-208-200)
SRE kit	PacBio (102-208-300)
SRE XL kit	PacBio (102-208-400)
1.5 mL DNA LoBind microcentrifuge tubes	Eppendorf (022431021)
200 µL wide bore pipette tips	USA Scientific (1011-8410)
Ethanol (96–100%)	
DI water	
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantification	Thermo Qubit 3.0, Qubit dsDNA BR Assay Kit

For all protocols

- Eppendorf DNA LoBind tubes (Eppendorf #022431021) are recommended for most library preparations.

Introduction

The short read eliminator kit family (SRE XS, SRE, SRE XL) can be used for rapid high-pass size selection of high molecular weight (HMW) DNA samples. This method can significantly enhance mean read length by progressively depleting short DNA up to 10 kb (SRE XS), 25 kb (SRE), or 40 kb (SRE XL) in length (Figure 1). Read length N50 can be increased by up to 10 – 30 kb depending on input sample quality. The kits use a centrifugation procedure similar to standard ethanol precipitation techniques.

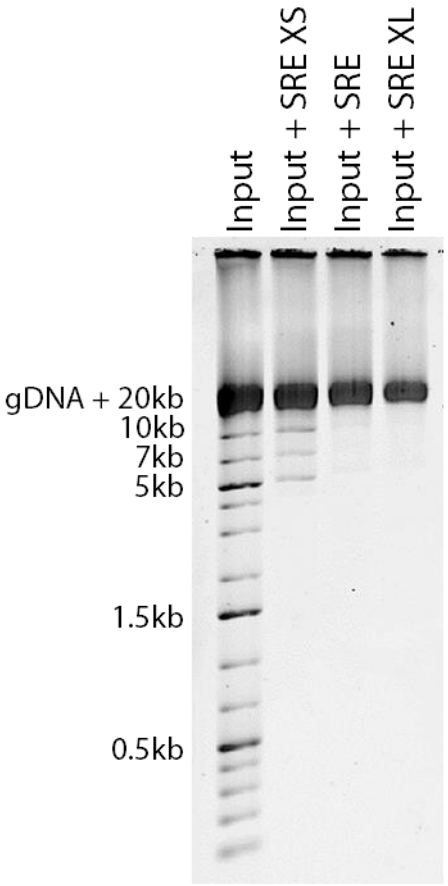


Figure 1. 1% Agarose gel separation of size-selected DNA with size cutoffs demonstrated using a spiked-in ladder (Thermo Scientific GeneRuler 1 kb Plus, #SM1334). Input is 50 ng/µL gDNA extracted from GM12878 cells using Nanobind® CBB kit + 20 ng/µL ladder.

Sample information and expected performance

The choice of kit to use should be based on the desired size selection performance and the quality of the input DNA as outlined in the table below. The stated recovery efficiencies will only be achieved when suitable quality input DNA is used at the appropriate Qubit DNA concentration.

Short read eliminator kit family				
Version	**DNA depletion range**	**Qubit DNA input**	**Recovery efficiency**	**Sample notes**
SRE XS	Progressive depletion: <10 kb Near complete depletion: <5 kb	25 – 150 ng/µL	50-90%	Suitable for sheared/fragmented DNA
SRE	Progressive depletion: <25 kb Near complete depletion: <10 kb	50 – 150 ng/µL	50-70%	Requires high quality HMW DNA (>48 kb)
SRE XL	Progressive depletion: <40 kb Near complete depletion: <10 kb	50 – 150 ng/µL	40-50%	Requires very high quality HMW DNA (>>48 kb)

The SRE XS kit should be used if the DNA sample is sheared/fragmented, has low concentration, or if very high recovery is needed. The SRE kit requires high quality HMW DNA where the majority of DNA is >48 kb. The SRE XL kit requires very high quality HMW DNA where the majority of DNA is >> 48 kb. Using a lower quality DNA sample than suggested will result in lower than expected recovery efficiency.

For the SRE XS kit, the DNA concentration must be between 25 – 150 ng/µL. For the SRE and SRE XL kits, the DNA concentration must be between 50 – 150 ng/µL. Using a lower than suggested DNA input concentration will result in lower than expected recovery efficiency. Using a higher than suggested DNA input concentration could impact size selection performance.

It is essential that DNA concentration is determined by Qubit or PicoGreen assay. Using concentrations derived solely from UV-Vis measurements will often result in low recovery as the DNA concentration will be over-estimated due to RNA that may also be present in the sample.

The DNA sample should be in TE buffer (pH 8), the supplied Buffer EB, or water. If the sample buffer differs significantly or contains high levels of salt, the size selection properties and recoveries may be affected.

HMW DNA size selection (all versions)

Each SRE kit progressively depletes short DNA beginning at its upper cutoff, with depletion efficiency increasing as the DNA gets shorter. Figure 2 illustrates the performance of the short read eliminator kit. DNA <10 kb in length is removed to levels that typically cannot be detected by gel or CE. DNA from 10 – 25 kb in length appears progressively removed.

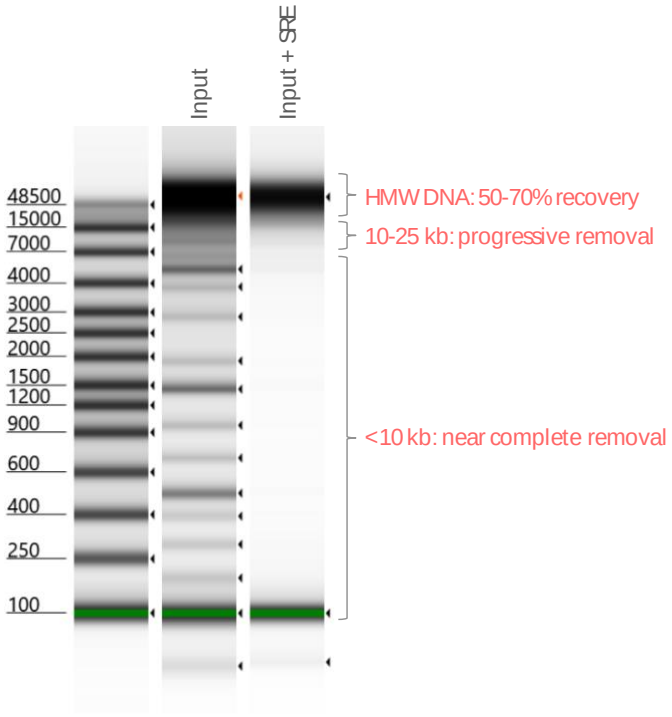


Figure 2. The SRE kit was used to size select HMW DNA from GM12878 cells that was isolated using the Nanobind CBB kit and spiked with a 1 kb DNA ladder.

The range for near complete depletion is estimated based on agarose gel and TapeStation analysis of HMW DNA samples spiked with ladders as shown in Figures 1 – 3. Due to the progressive depletion, size selection is not always apparent when samples containing DNA smears are analyzed by gel or CE. The upper cutoff for progressive depletion is estimated based on comparison of read length distributions between size selected and non-size selected samples as shown in Figure 5.

The overall recovery efficiency is highly dependent on the quality and concentration of the input DNA. The SRE XS kit has the highest overall recoveries and is the most tolerant of sheared/fragmented DNA or low input concentrations. The SRE and SRE XL kits require high quality HMW DNA. If the samples contain large amounts of fragmented DNA or if the DNA is not sufficiently HMW, the recovery efficiency will be lower than expected. Figure 3 shows the expected recovery efficiency for Nanobind extracted HMW gDNA samples that were size selected using each of the SRE kits.

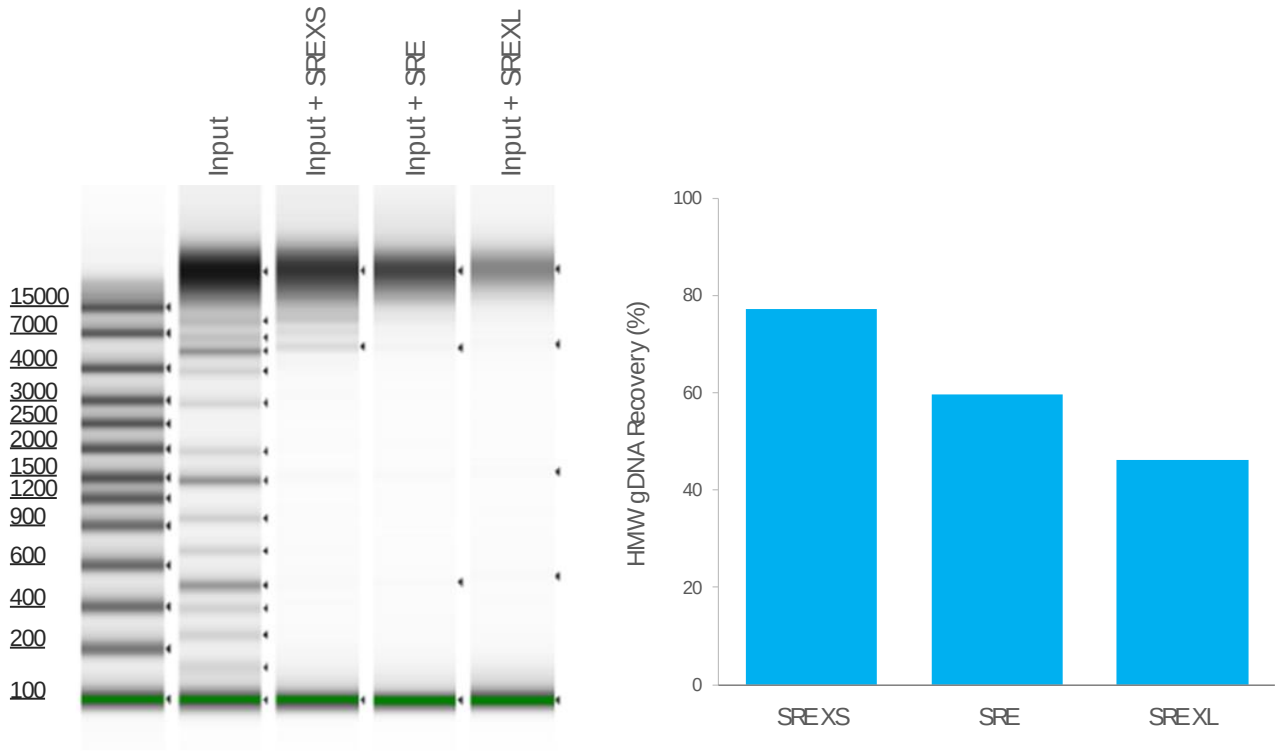


Figure 3. The sample from Figure 1 was analyzed on Agilent Tapestation 4200. HMW gDNA recoveries were measured using the same input DNA without the ladder.

Sheared/fragmented DNA size selection (SRE XS only)

For sheared or fragmented DNA samples, only the SRE XS kit should be used. Use of the SRE or the SRE XL kits on sheared/fragmented DNA samples will likely result in very low recovery efficiency.

Figure 4 shows size selection and recovery data from DNA samples that were sheared down to 10, 20, and 30 kb using Diagenode Megaruptor 3. DNA input concentration down to 25 ng/µL can be used for all samples except the 10 kb sample, which requires 50 ng/µL to obtain suitable recovery.

The SRE XS can also be used on long PCR amplicons.

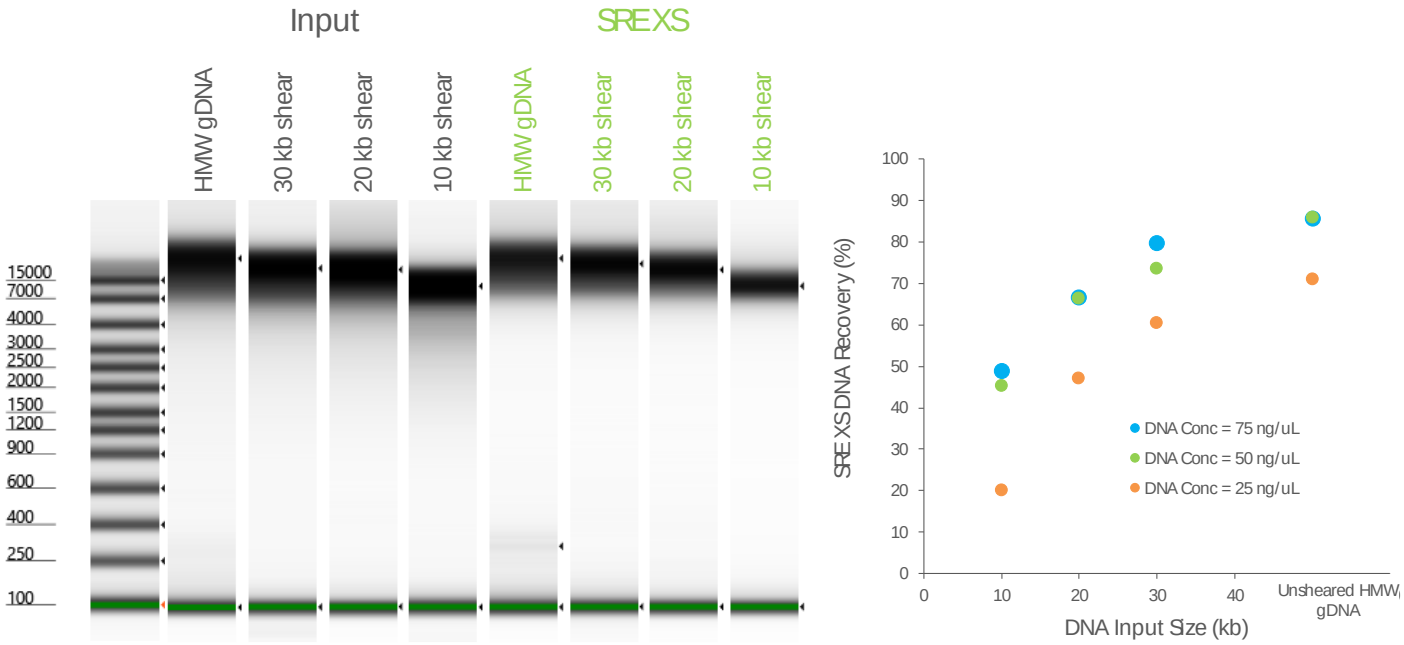


Figure 4. gDNA was extracted from GM12878 cells using the Nanobind CBB kit, diluted to 100 ng/µL, and sheared to 10, 20, or 30 kb using Megaruptor 2. The samples were then size selected using the SRE XS Kit and analyzed on Agilent Tapestation 4200. Recovery efficiency decreases with DNA length but is >50% for all samples at input concentrations ≥50 ng/µL. 25 ng/µL can be used for all samples except the 10 kb sample, unless low recovery efficiency can be tolerated.

PacBio Sequel IIe sequencing

HMW DNA was extracted from degraded human blood using the Nanobind CBB kit (102-301-900) and from mistletoe leaves using the Nanobind plant kit and then sequenced on the PacBio Sequel IIe instrument using the SMRTbell prep kit 3.0. Size selection of the HMW DNA was performed before shearing using the SRE kit just before library preparation increased mean read lengths by depleting DNA < 10 kb.

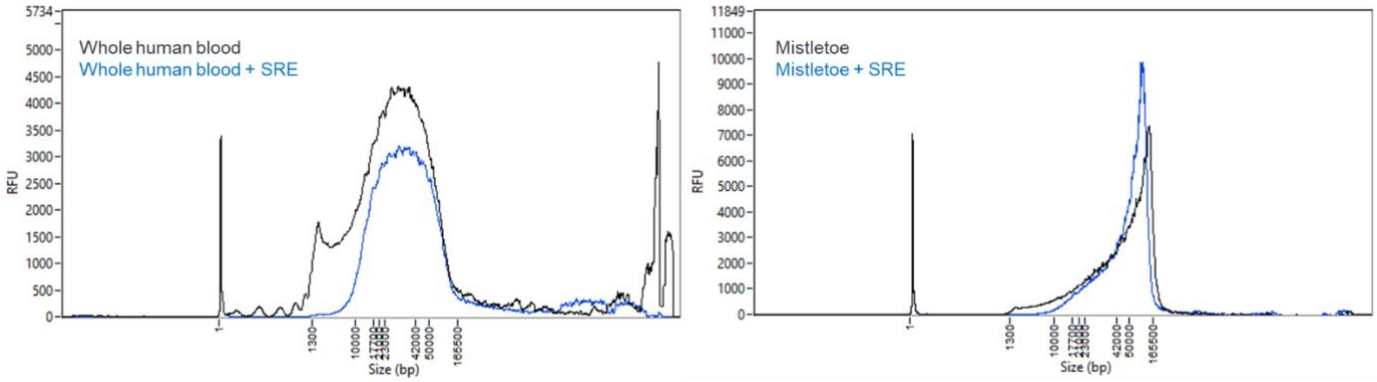


Figure 5. gDNA was extracted from degraded whole human blood using the Nanobind CBB big DNA kit and from mistletoe leaves using the Nanobind plant kit. The DNA was diluted to 50 ng/µL, size selected using the SRE kit and analyzed on Femto Pulse. Typical recoveries are 50—70% , and the depletion of DNA < 10 kb can be seen clearly in the eletropherograms.

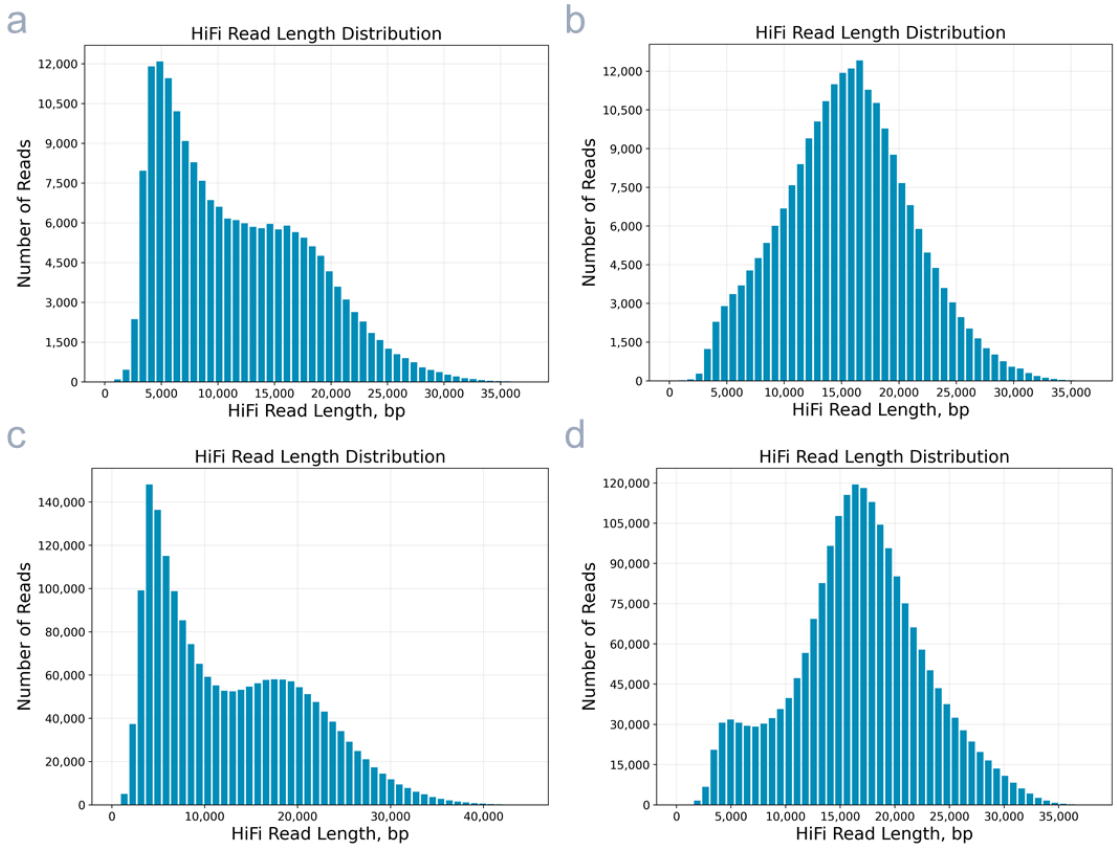
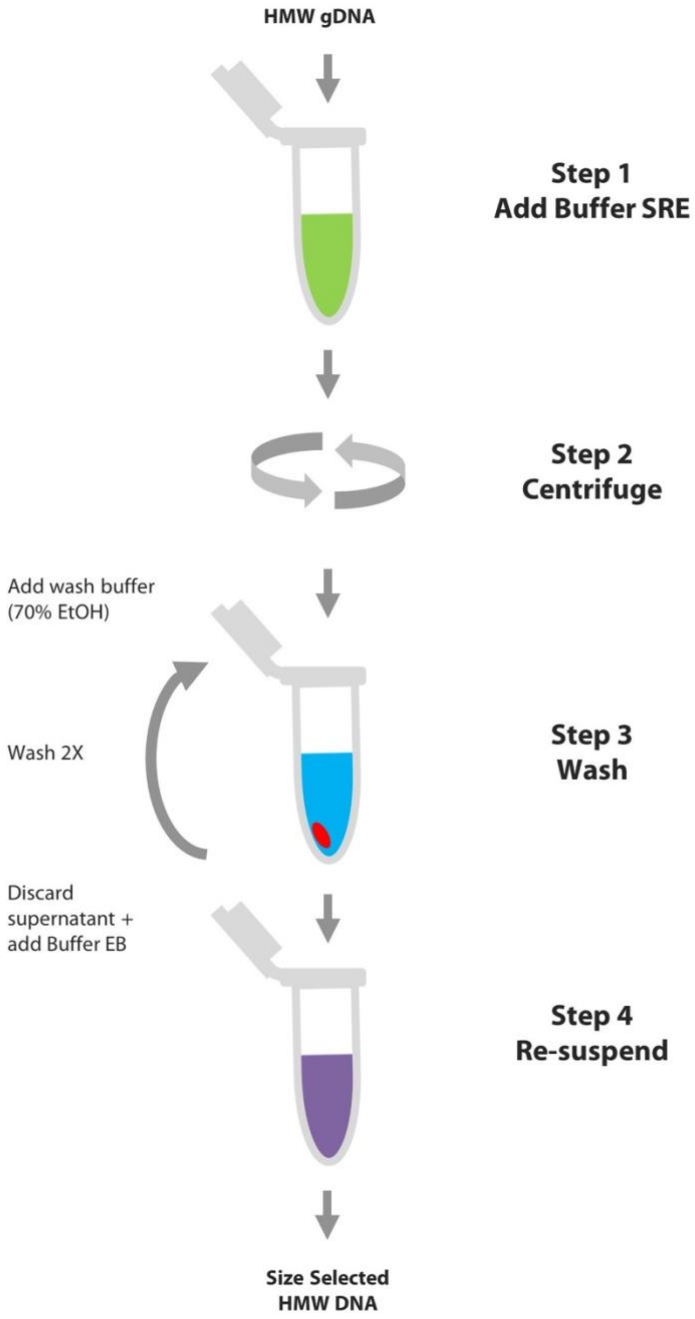


Figure 6. gDNA from Figure 5 was sheared, and libraries were prepared using the SMRTbell prep kit 3.0 and then sequenced on the Sequel IIe system. HiFi read length distributions show that size selection using the SRE kit on unsheared HMW DNA removes short reads, leading to higher mean read lengths: a) Whole human blood; b) Whole human blood + SRE ; c) Mistletoe leaves; d) Mistletoe leaves + SRE.

Sequel IIe			
Sample Type	**Size selection**	**SMRTbell GQN(10k)**	**Mean read length (bp)**
Human whole blood	None	7.9	11,634
Human whole blood	Short read eliminator	9.3	15,516
Mistletoe leaves	None	9.2	12,227
Mistletoe leaves	Short read eliminator	9.6	18,906

Table 1. gDNA was extracted using the Nanobind CBB big DNA kit or Nanobind plant kit, size selected using the short read eliminator kit, and sequenced on PacBio Sequel IIe.

Workflow



Processing tips

Pipetting

Load tube into centrifuge with the hinge facing toward the outside of the rotor. This will help to avoid disturbing the pellet if it cannot be seen. After centrifugation, the DNA pellet will have formed on the bottom side of the microcentrifuge tube under the hinge region. Pipette on opposite side towards the thumb lip to avoid disturbing the pellet as shown in Figure 7.

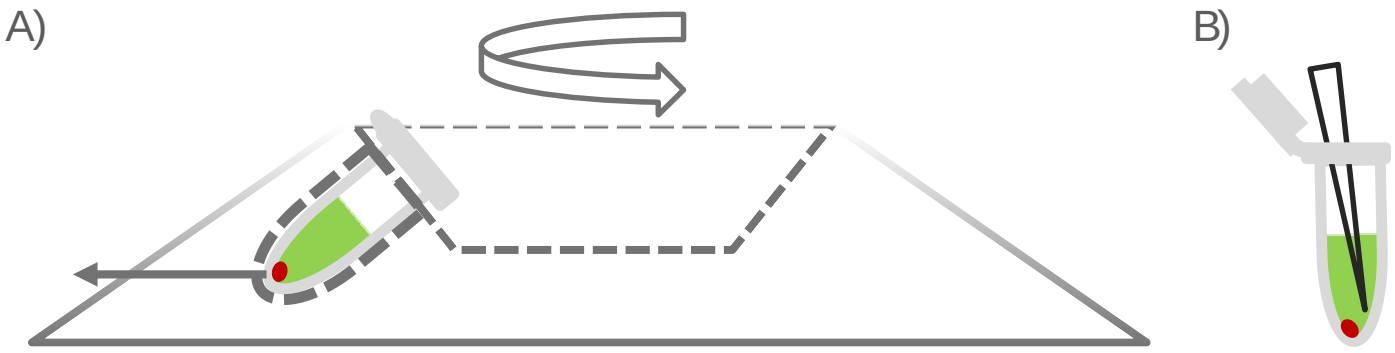


Figure 7. A) Note orientation of tube in centrifuge. Pellet will form on side of the tube facing outwards, in this case underneath the hinge region. B) Pipette from opposite side of tube on the thumb lip side to avoid disturbing pellet. Pellet may not be visible.

Heterogeneity and viscosity

Recovery efficiency and size selection performance of the short read eliminator kits depends on the input DNA being homogeneous and fully in solution. HMW DNA can sometimes be difficult to re-solubilize after extraction and results in a jelly-like, inhomogeneous sample. Such samples will result in low yields and carry-over of short DNA if used with the short read eliminator kits. If the HMW DNA sample is inhomogeneous or contains viscous jellies, we recommend needle shearing with 5-10X with a 26G needle and then allowing the DNA to rest at RT overnight before beginning size selection. Sample homogeneity can be evaluated by performing triplicate concentration measurements and verifying that the concentration CV is <20%.

Size selection protocol for SRE and SRE XL

The following protocol details size selection of HMW. For HiFi sequencing, size selection should be performed on gDNA prior to shearing and library preparation. The input HMW DNA should have length >48 kb and Qubit DNA concentration >50 ng/µL. **Always use wide bore pipettes.**

HMW gDNA

1. Adjust the DNA sample to a total volume of 60 µL and a Qubit DNA concentration of between 50 – 150 ng/µL. Pipette sample into a 1.5 mL Eppendorf DNA LoBind tube.
   - This concentration MUST be measured using Qubit dsDNA Broad Range Assay or equivalent.
   - Dilute sample using TE buffer (pH 8), Buffer EB, or water.
2. Add 60 µL of Buffer SRE or Buffer SRE XL to the sample. Mix thoroughly by gently tapping the tube or by gently pipetting up and down.
3. Load tube into centrifuge with the hinge facing toward the outside of the rotor.
4. Centrifuge at 10,000 x g for 30 mins at room temperature (RT).
   - If using a centrifuge with temperature control (i.e. cooling function), turn this function off by setting the temperature to 29°C.
5. Carefully remove supernatant from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 7).
   - The DNA pellet will have formed on the bottom of the tube under the hinge region.
6. Add 200 µL of the 70% EtOH wash solution to tube and centrifuge at 10,000 x g for 2 mins at RT.
   - Do not tap or mix after adding 70% EtOH. Place tube directly into centrifuge.
7. Carefully remove wash solution from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 7).
8. Add 50 - 100 µL of Buffer EB to the tube and incubate at room temperature for 20 minutes. Buffer volume may be adjusted to achieve desired concentration.
9. After incubation, gently tap the tube to ensure that the DNA is properly re-suspended and mixed.
10. Analyze the recovery and purity of the DNA by NanoDrop and Qubit.

Quick tip

Using concentrations derived from UV-Vis measurements without accounting for RNA concentrations will adversely affect yields.

Quick tip

The DNA pellet may not be visible. Placing the tube and pipetting in the directed orientations will prevent accidentally aspirating the DNA pellet.

Quick tip

Longer DNA can take more time to re-suspend. Heating to 50°C or eluting for more time can help increase recoveries.

Size selection protocol for SRE XS

The following protocol details size selection of HMW gDNA or fragmented DNA. For HiFi sequencing, size selection should be performed on gDNA prior to shearing and library preparation. The Qubit DNA concentration should >25 ng/µL. **Always use wide bore pipettes.**

HMW gDNA or sheared/fragmented DNA

1. Adjust the DNA sample to a total volume of 60 µL and a Qubit DNA concentration of between 25 – 150 ng/µL. Pipette sample into a 1.5 mL Eppendorf DNA LoBind tube.
   - This concentration MUST be measured using Qubit dsDNA Broad Range Assay or equivalent.
   - Dilute sample using TE buffer (pH 8), Buffer EB, or water.
2. Add 60 µL of Buffer SRE XS to the sample. Mix thoroughly by gently tapping the tube or by gently pipetting up and down.
3. Load tube into centrifuge with the hinge facing toward the outside of the rotor.
4. Centrifuge at 10,000 x *g* for 30 mins at room temperature (RT).
   - If using a centrifuge with temperature control (i.e. cooling function), turn this function off by setting the temperature to 29°C.
5. Carefully remove supernatant from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 7).
   - The DNA pellet will have formed on the bottom of the tube under the hinge region.
6. Add 200 µL of the 70% EtOH wash solution to tube and centrifuge at 10,000 x *g* for 2 mins at RT.
   - Do not tap or mix after adding 70% EtOH. Place tube directly into centrifuge.
7. Carefully remove wash solution from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 7).
8. Add 50 - 100 µL of Buffer EB to the tube and incubate at room temperature for 20 minutes. Buffer volume may be adjusted to achieve desired concentration.
9. After incubation, gently tap the tube to ensure that the DNA is properly re-suspended and mixed.
10. Analyze the recovery and purity of the DNA by NanoDrop and Qubit.

Quick tip

Using concentrations derived from UV-Vis measurements without accounting for RNA concentrations will adversely affect yields.

Quick tip

The DNA pellet may not be visible. Placing the tube and pipetting in the directed orientations will prevent accidentally aspirating the DNA pellet.

Quick tip

Longer DNA can take more time to re-suspend. Heating to 50°C or eluting for more time can help increase recoveries.

Troubleshooting guide

Recovery is poor:

- **Highly fragmented gDNA.** Recovery will be low if DNA is not HMW. Verify that a significant fraction of the input DNA is >48 kb by using PFGE or capillary electrophoresis.
- **Low input DNA concentration.** Recovery will be low if dsDNA concentration is <50 ng/µL. Verify the input gDNA concentration using Qubit dsDNA broad range assay or equivalent. Using concentrations derived solely from UV-Vis measurements will often result in low recovery as the estimated DNA concentration will not account for RNA that is also present in the solution. Try increasing concentration of input DNA up to the maximum of 150 ng/µL.
- **Incorrect centrifugation speed.**
- **Incorrect centrifugation temperature.** Recovery will be impacted if centrifugation is performed at low temperature (e.g. 4°C). Verify that the centrifuge is not cooling by turning off cooling or setting the temperature above ambient (e.g. 29°C).
- **Heterogeneous input sample.** If input sample is heterogeneous and contains fractions of DNA that are not fully solubilized, recovery will be affected. Verify homogeneity by pipetting to ensure that no viscous jellies exist in the sample. Homogeneity can also be determined by performing triplicate concentration measurements and verifying that the CV <20%. If the sample fails these tests, needle shear the input DNA 10X using a 26G needle or pipette mix 10X using a standard P200 pipette and allow to rest overnight at RT before proceeding.
- **Non-standard DNA buffer.** This method has only been tested using DNA in solubilized in TE buffer (pH 8), Buffer EB, or water. If the DNA sample contains high levels of contaminants or salts, recovery may be affected.
- **Handling error.** The DNA pellet is often invisible. If the pellet is disturbed during the wash steps, it is possible to accidentally aspirate it into the pipette tip. Ensure that proper care is taken with tube orientation during centrifuge and pipetting steps such that pipetting is always performed on the opposite side of the tube from the pellet.

Cutoff seems too low/too high:

- **Non-standard DNA buffer.** This method has only been tested using DNA in water, TE buffer, or Buffer EB. If the DNA sample contains high levels of contaminants or salts or compounds that affect DNA solubility/precipitation, size selection performance may be affected.